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Abstract: Hypertension in animal models and in humans is associated with a decreased vasodilator response to acetylcholine which causes vascular relaxation by release of endothelium-derived relaxing factor from the endothelium. Since lowering of blood pressure, particularly with angiotensin converting enzyme inhibitors, improved the response to acetylcholine we investigated the effects of brachial artery infusions of ascending dosages of actetylcholine on forearm blood flow before and after 5 months of therapy with the angiotensin converting enzyme inhibitor, cilazapril, in 10 patients with mild to moderate primary hypertension. Cilazapril decreased blood pressure from 150.8 ± 14.4/98.9 ± 4.3 mmHg during placebo to 138.8 ± 15.6/88.6 ± 8.9 mmHg ($P < 0.01$). Brachial artery acetylcholine infusions increased forearm blood flow from 2.95 ± 1.5 to a maximum of 22.8 ± 11.5 ml.min−1.100 ml−1 forearm tissue and decreased forearm vascular resistance from 48.1 ± 34.1 to 6.9 ± 6.9 units before cilazapril. This response did not change after cilazapril therapy. Our findings in patients with primary hypertension, therefore, do not support the concept that angiotensin converting enzyme inhibition influences endothelium-dependent vascular relaxation to acetylcholine to a significant degree. Whether this lack of effect on endothelial vasodilator function is specific for the vascular bed chosen for study or whether it represents a fundamental difference between animal models and human hypertension remains an important issue to be clarified

DOI: https://doi.org/10.1093/eurheartj/14.suppl_c.5

European Heart Journal (1993) **14** (*Supplement C*), 5–9

Effects of angiotensin converting enzyme inhibition on endothelial vasodilator function in primary human hypertension

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KEY WORDS: Angiotensin-converting enzyme inhibition, cilazapril, endothelium, acetylcholine, forearm blood flow, hypertension

Hypertension in animal models and in humans is associated with a decreased vasodilator response to acetylcholine which causes vascular relaxation by release of endothelium-derived relaxing factor from the endothelium. Since lowering of blood pressure, particularly with angiotensin converting enzyme inhibitors, improved the response to acetylcholine we investigated the effects of brachial artery infusions of ascending dosages of actetylcholine on forearm blood flow before and after 5 months of therapy with the angiotensin converting enzyme inhibitor, cilazapril, in 10 patients with mild to moderate primary hypertension. Cilazapril decreased blood pressure from 150·8 ± 14·4/98·9 ± 4·3 mmHg during placebo to 138·8 ± 15·6/88·6 ± 8.9 mmHg ($P < 0·01$). Brachial artery acetylcholine infusions increased forearm blood flow from 2·95 ± 1·5 to a maximum of 22·8 ± 11·5 ml.min^{-1}.100 ml^{-1} forearm tissue and decreased forearm vascular resistance from 48·1 ± 34·1 to 6·9 ± 6·9 units before cilazapril. This response did not change after cilazapril therapy. Our findings in patients with primary hypertension, therefore, do not support the concept that angiotensin converting enzyme inhibition influences endothelium-dependent vascular relaxation to acetylcholine to a significant degree. Whether this lack of effect on endothelial vasodilator function is specific for the vascular bed chosen for study or whether it represents a fundamental difference between animal models and human hypertension remains an important issue to be clarified.

Introduction

Since the landmark description by Furchtgott and Zawadski[1] that the endothelium is obligatory for the vasodilator effects of acetylcholine, it has been increasingly recognised that the endothelial cell monolayer, by virtue of its location, not only separates blood from underlying vascular muscle and preserves blood fluidity, but is also important in the regulation of vascular tone by synthesizing and releasing highly potent, vasoactive compounds[2,3]. The mechanism responsible for the vasodilator effect of acetylcholine has been mostly clarified and most of the activity of what has been termed endothelium-derived relaxing factor (EDRF) can be attributed to endothelial generation of nitric oxide or a substance that spontaneously generates nitric oxide[4] from l-arginine[5]. Moreover, it has become clear that basal release of EDRF, both in animal experiments[6,7] and healthy man[8], is involved in the regulation of vascular tone. In addition to modulating vascular muscle tone, the endothelium appears to be a key element in the early phases of the development of atherosclerosis[9]. The direct study of endothelial integrity is difficult in man, but endothelial function can be assessed as vasodilator response to muscarinergic receptor agonists such as acetylcholine. Thus, it has been demonstrated that the vascular action of acetylcholine is independent of prostacyclin production or inhibition of adrenergic neurotransmission. Accordingly, the vascular effects of acetylcholine can be ascribed to release of endothelium-derived relaxing factor[10] and there is evidence that e.g. hypercholesterolaemia is associated with endothelial dysfunction in human coronary conduit and resistance vessels in vivo which precedes angiographically visible atherosclerotic lesions in large coronary arteries[11]. Similar findings were made in forearm resistance vessels[12]. Likewise, hypertension as another important risk factor for the development of atherosclerosis, is associated with a decreased vasodilator response in forearm resistance vessels to acetylcholine[10,13]. Because alterations of endothelial function and morphology appear to coincide[14] and may be markers of early atherosclerotic vascular changes, the effects of treatment on acetylcholine-mediated release of endothelium-derived relaxing factor, as a marker of endothelial function, are worth considering. Because of experimental evidence that angiotensin converting enzyme inhibitors, but not hydralazine, improved endothelial vasodilator function in spontaneously hypertensive rats[14], we investigated the effects of cilazapril on the vasodilator response to acetylcholine in patients with primary hypertension.

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Subjects and methods

Ten patients with a history of mild to moderate hypertension (6 male, 4 female, aged 30 to 55 (mean 46·8 ± 7.2) years) consented to participate in the study which was approved by the hospital's ethics committee. Secondary forms of hypertension were ruled out by a standard work-up[15]. None of the patients had been treated with calcium

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channel blockers or angiotensin converting enzyme inhibitors in the preceding 3 months.

STUDY DESIGN

The study was designed as a single-blind, placebo-controlled study lasting 24 weeks. All previous medication was discontinued and the patient was given placebo tablets identical in appearance to the active compound. Blood pressure was always measured in the morning and the patients were instructed not to take their morning dose on those days. When casual diastolic blood pressure was greater than 95 mmHg after 3 weeks of placebo once daily, the investigation of endothelial function was scheduled 7 days later with the patient still taking placebo. After completing the first haemodynamic study, patients were given cilazapril 5 mg once daily for one week and after ensuring that no adverse clinical or biochemical effects had occurred, the dose was titrated to 5 mg twice daily for the remainder of the 20 weeks active treatment period. Additional clinic visits were scheduled after 4, 8 and 12 weeks and at the end of the last week of the study period when the study of endothelial vasodilator function was repeated.

METHODS

Compliance was assessed by counting the number of returned tablets and was assumed to be good when the number of tablets taken was within 20% of the theoretical value for the respective treatment interval. During clinic visits, blood pressure was assessed using a standard mercury sphygmomanometer and heart rate was counted from the radial pulse.

All haemodynamic studies were performed in the morning with the subjects recumbent and comfortably resting after a light breakfast in a quiet, air-conditioned room at an ambient temperature of 20–22°C. Under local anaesthesia (lidocaine 1%) an 18 gauge catheter (Abbocath-T, Abbott, Sligo, Ireland) was inserted into the left brachial artery for regional drug infusion and recording of arterial pressure using a Statham P23 Pb pressure transducer. The subjects were allowed to rest for 30 min following completion of instrumentation until basal forearm blood flow, intra-arterial blood pressure and heart rate were recorded. Next, sodium nitroprusside (0·6 μg.min.$^{-1}$.100 ml^{-1} forearm tissue) was infused in the brachial artery and forearm blood flow measured in the third minute of infusion. After return of forearm blood flow to baseline (20 to 30 min) the resting flow was again measured. Finally, acetylcholine (0·8, 10, 40 and 160 μg.min^{-1}.100 ml^{-1}) was infused over 3 min each[10] and forearm blood flow measured in the last minute of each infusion. Blood pressure and heart rate were recorded immediately after completion of each infusion.

Forearm blood flow was measured bilaterally by venous occlusion plethysmography[16]. In short, a mercury in-silastic strain gauge was placed at the upper third of the forearm which rested comfortably on a support slightly above the level of the heart. The strain gauge was coupled to an electronically calibrated plethysmograph (EC3, Hokanson, Watassah, WA, U.S.A.). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mmHg by a rapid cuff inflator (EC10, Hokanson). The hand was excluded from the circulation by inflating a paediatric blood pressure cuff placed around the wrist to 50 mmHg above systolic pressure one minute prior to and during the forearm blood flow measurements, in order to eliminate the unpredictable influence of arterio-venous shunts in the hand. Experiments were done on the left (experimental) forearm, while blood flow measurements on the right (control) arm served as continuous control. Determinations of forearm blood flow were made by analysing four to six consecutive recordings using a digitizing board and suitably programmed computer. The mean value was taken for statistical evaluation. Forearm vascular resistance was calculated by dividing mean arterial pressure by forearm blood flow and is expressed as arbitrary units. The electrocardiogram was monitored throughout the study.

STATISTICAL ANALYSIS

Results are expressed as means ±SD. One factor analysis of variance was used to test for differences attributable to the different drugs. Responses to acetylcholine infusions and the influence of cilazapril were analysed using profile analysis for repeated measures. The paired and unpaired Student's t-test and linear regression analysis were employed as appropriate. A two-tailed *P*-value of less than 0·05 was considered to indicate a significant difference. All calculations were performed using the StatView II (Abacus Inc, Berkeley, CA, U.S.A.) statistical program.

Results

Angiotensin converting enzyme inhibition by cilazapril was well tolerated and causal sitting blood pressure decreased from 150·8 ± 14·4/98·9 ± 4·3 mmHg during placebo treatment to 138·8 ± 15·6/88·6 ± 8·9 mmHg after 20 weeks of cilazapril ($P < 0·01$). Similar decreases were observed for intra-arterially recorded blood pressures and heart rate remained unchanged.

The results of the studies of endothelial dependent (acetylcholine) and independent (sodium nitroprusside) vasodilatation are depicted in Figs 1 and 2. Sodium nitroprusside increased forearm blood flow during placebo from 2·7 ± 1·0 to 11·1 ± 5·4 ml.min.$^{-1}$.100 ml^{-1} and decreased forearm vascular resistance from 43·1 ± 21·6 to 12·0 ± 9·0 units. As shown in Fig. 1, the effect of sodium nitroprusside on forearm haemodynamics remained essentially unchanged after cilazapril therapy. Brachial artery acetylcholine infusions increased forearm blood flow from 2·95 ± 1·5 to a maximum of 22·8 ± 11·5 ml.min.$^{-1}$.100 ml^{-1} and decreased forearm vascular resistance from 48·1 ± 34·1 to 6·9 ± 6·9 units. As shown in Fig. 2, this response did not differ after cilazapril therapy. Individual changes in vascular responsiveness to acetylcholine were not related to age, height of blood pressure before treatment or cilazapril-induced decreases of either systolic or diastolic blood pressure. Increases in forearm blood flow to sodium nitroprusside and acetylcholine on placebo were significantly correlated with values observed after cilazapril therapy ($r = 0·61$ and $r = 0·68$, both $P < 0·05$).

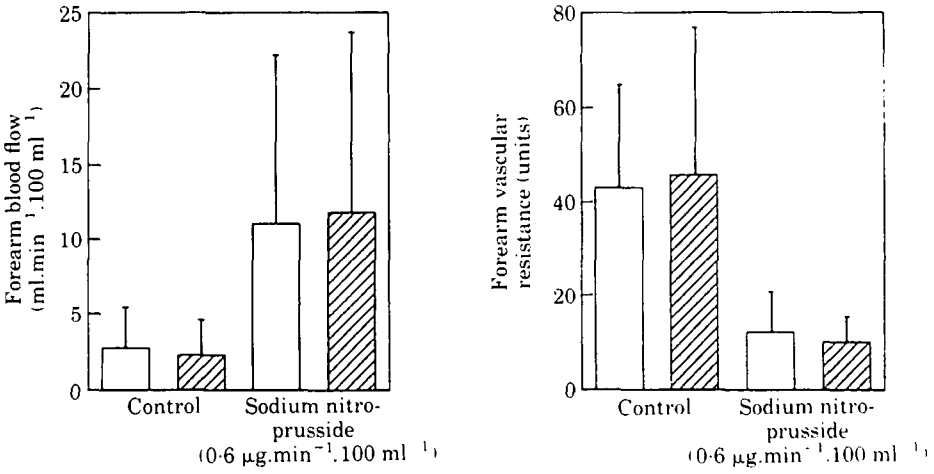

Figure 1 Response of forearm resistance vessels to brachial artery infusion of sodium nitroprusside in 10 hypertensive patients. The response of forearm blood flow is depicted in the left panel, the reduction of calculated forearm vascular resistance in the right panel. Open bars represent results obtained at the end of the placebo period and hatched bars after 5 months of therapy with cilazapril 5 mg b.i.d. Values are means ± standard deviation.

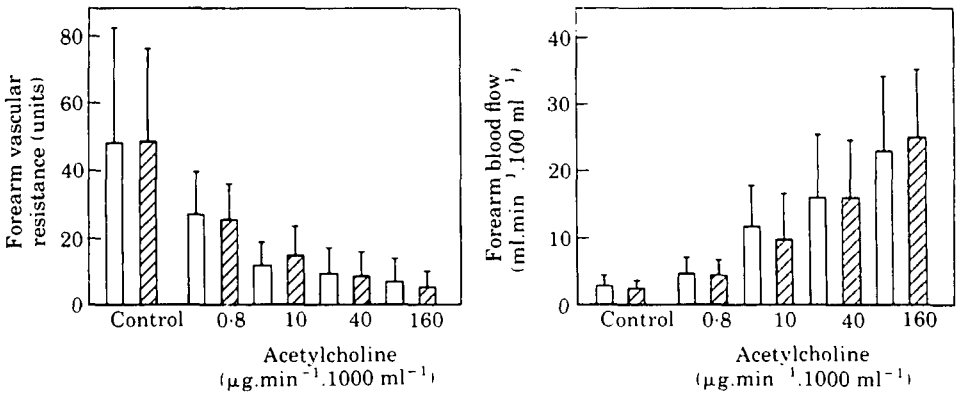

Figure 2 Response of forearm resistance vessels to brachial artery infusion of ascending dosages of acetylcholine in 10 hypertensive patients. The response of forearm blood flow is depicted in the left panel, the reduction of calculated forearam vascular resistance in the right panel. There was no difference in the vascular response to acetylcholine when comparing results obtained at the end of the placebo period (open bars) and those after 5 months of therapy with cilazapril 5 mg b.i.d. (hatched bars). Values are means ± standard deviation.

Discussion

Arterial hypertension in humans and animal models of hypertension is associated with structural and functional changes of the arterial system, including the resistance vessels which are responsible for the main haemodynamic disturbance found in hypertension, e.g. elevated peripheral vascular resistance[17]. Folkow and co-workers[17] were the first to draw attention to the finding that hypertensive resistance vessels have an altered structural design due to an increase in medial thickness resulting in an increased wall to lumen ratio, rendering the vessels more responsive to vasoconstrictor stimuli. Recent evidence suggests that actual medial hypertrophy in hypertensive resistance arteries can only explain a fraction of the encroachment of the lumen observed in stroke prone spontaneously hypertensive rats and that the greater part of this disturbance is due to a reduction in external diameter, a finding termed 'remodelling'[18]. Similar considerations appear to apply to human primary hypertension[19].

Several functional aspects of regulation of vascular resistance also appear to be disturbed in human hypertension, including a greater sympathetic nervous system contribution to resting arteriolar tone[16,20], enhanced calcium-influx dependent vasoconstriction[21,22] and, more recently, impaired vasodilatation in response to muscarinergic stimulation[10,13]. The latter response in the human forearm is mediated by endothelium-derived relaxing factor and not by interference with neural release of catecholamines or endothelial release of prostacyclin[10]. Therefore, human

hypertensive resistance vessels appear to resemble animal models of hypertension in which a diminished vasodilator response of large conduit arteries to muscarinergic stimulation is commonly observed in vitro[23–27]. In contrast to findings in humans, microvascular relaxation in response to acetylcholine was normal in salt-sensitive hypertensive Dahl rats[28] and rabbits with cellophane wrap-induced hypertension[29]. Interpretation of results is further complicated by the finding that the degree of endothelial dysfunction varied in differing vascular beds in spontaneously hypertensive rats[30]. Interestingly, antihypertensive therapy and reversal of hypertension[26,31] normalized disturbed endothelial vasodilator function in animal models of hypertension. The observation that the angiotensin converting enzyme inhibitor cilazapril, but not the direct acting vasodilator hydralazine[14], improved endothelium-mediated vasodilator responses to muscarinergic stimulation in rat models of hypertension, raised the possibility that mechanisms other than blood pressure lowering per se are important in that respect. In view of the proposed key role of the endothelium in the development of atherosclerosis and its vascular complication[9], and considering that an impairment of acetylcholine-induced coronary artery dilation is one of the earliest findings in subjects with risk factors for, but no angiographic evidence of, coronary atherosclerosis[11], the normalization of endothelial function by angiotensin converting enzyme inhibition in animals might point to a potentially important aspect of antihypertensive therapy which so far has received little attention in humans.

The present study investigated the effects of 5 months antihypertensive monotherapy with cilazapril on acetylcholine-mediated relaxation of forearm resistance vessels which exhibit reduced responsiveness to, or a diminished release of, endothelium-derived relaxing factor in response to muscarinergic stimulation[10,13]. Cilazapril effectively lowered blood pressure, confirming its antihypertensive properties when given in monotherapy[32,33]. Despite the pronounced decrease in blood pressure, we were unable to find an improvement in the vascular response to acetylcholine. Responses to both endothelium-dependent, e.g. acetylcholine, and endothelium-independent, e.g. sodium nitroprusside, relaxation obtained before and after cilazapril therapy were closely correlated attesting to the reproducibility of the method employed. Therefore, these results are at variance with previous reports of the effects of antihypertensive therapy on endothelial function in various animal models of hypertension[14,26,31].

There is no ready explanation for this discrepancy, but several aspects need to be considered. Thus, therapy lasted 5 months which may not be enough to reverse potential changes brought about by long-standing hypertension. However, in spontaneously hypertensive rats, cilazapril improved acetylcholine-mediated vasodilatation after 4 days of therapy, suggesting that endothelial function can be influenced within a short time span[14]. The disturbed endothelial response to acetylcholine in hypertensive Dahl rats has also been found to depend upon the magnitude of blood pressure elevation[25]. Accordingly, it might be argued that in patients with more severe hypertension a more pronounced endothelial impairment might have been improved by therapy. On the other hand, captopril, but not hydralazine, had a marked potentiating effect on acetylcholine-induced relaxations of aortic rings from normotensive rats[34]. Interestingly, the angiotensin converting enzyme inhibitor, enalapril, did not affect the vascular acetylcholine response in that study. Thus, it appears unlikely that the degree of hypertension was a deciding factor in the inability of cilazapril to influence the response of the forearm vasculature to acetylcholine. More obvious is the fact that most of the animal and in-vitro data were obtained in vascular preparations from large conduit arteries, while the present data reflect the behaviour of resistance vessels mostly supplying skeletal muscle. This may, of course, reflect a fundamental difference in the response of the endothelium in different sections of the arterial tree to lowering of blood pressure. Finally, species differences regarding their ability to change endothelial function in response to antihypertensive therapy cannot be excluded as an important factor for the discrepancy between our findings and animal experiments.

In conclusion, our findings obtained in vivo in patients with primary hypertension do not support the concept that angiotensin converting enzyme inhibition, despite a considerable decrease in blood pressure, influences endothelium-dependent vascular relaxation to acetylcholine to a significant degree. Whether this lack of effect on endothelial vasodilator function is specific for the vascular bed chosen for study, e.g. mostly resistance vessels supplying forearm skeletal muscle, or whether it represents a fundamental difference between animal models and human hypertension remains an important issue to be clarified.

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